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ENVIRONMENTAL

Electroanalytical Detection of the Pesticide Paraquat by Batch Injection Analysis

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Abstract: The Batch-Injection Analysis (BIA) technique has been applied to the electroanalytical detection of the herbicide paraquat by square wave voltammetry (SWV) during sample injection. The results obtained showed that the herbicide can be detected at $\mu g\, l^{-1}$ levels with small injection volumes ($<100\ \mu l$). The time of each measurement was less than two seconds. The BIA method presents many advantages such as being extremely fast, with high reproducibility, good sensitivity and simple without pre-addition of or changing the supporting electrolyte.

Keywords: Batch-injection analysis, paraquat pesticide, square wave voltammetry

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INTRODUCTION

Batch injection analysis (BIA) has, since its inception (Wang and Taha 1991) represented an innovative injection approach for performing rapid electroanalysis. The method involves the injection of a small volume of a liquid sample directly over a detector that is immersed in a large volume of blank solution. The potentialities and applications of BIA have been recently reviewed (Quintino and Angnes 2004), showing that it can be used with a large variety of detectors, such as electrochemical, optical and thermal.

Electrochemical BIA has been used in a variety of applications. Some of the most frequent of these have involved the sensing of traces of toxic metal ions, for example (Brett et al. 1996a; Brett et al. 1996b; Brett et al. 1999b Fungaro and Brett, 2000). Other substances have included ascorbate (Wang and Taha 1991; De Donato Pedrotti and Gutz 1999), pharmaceuticals such as acetaminophen (Quintino et al. 2002) and haemoglobin (Brett et al. 1999c). However, until the present and to our knowledge it has not been used to detect pesticides.

In the electrochemical BIA technique, the detector is a disc electrode immersed in electrolyte solution and injection is carried out directly over the centre of the disc. Electrochemical BIA presents a number of advantages from the analytical point of view such as (Brett et al. 1995; Brett 1999a): small sample injection volume ($\leq$100 μl which reduces the amount of adsorption blocking of the electrode surface), high reproducibility and sensitivity, real time and extremely fast analysis, the possibility of making many successive determinations without changing the supporting electrolyte as well as a special attraction for environmental monitoring due to the possibility of the use of portable instruments for *in situ* analysis. It is possible to carry out a square wave or cyclic voltammetric scan either during or immediately after the period of injection (Brett et al. 1994).

Paraquat (PQ), 1,1-dimethyl-4,4 bipyridinium dichloride, also known as methylviologen, is one of the most used pesticides in over 130 countries around the world. It is a pesticide of toxicological class I, i.e., extremely dangerous for human health. Pesticides are associated with oxidative stress (Abdollahi et al. 2004) and paraquat has been suggested to induce pathogenesis of dopaminergic neurons though oxidative stress (Yang and Tiffany-Castiglioni 2005). The main environmental problems in the uncontrolled uses of this pesticide are attributed to its high persistence and water solubility, more than 600 g l^{-1} (Extoxnet, 1996).

Paraquat can be detected by many different analytical techniques such as liquid and gas chromatography (Munch and Bashe 1997; Corasaniti and Nisticò 1993; Castro et al. 2000), spectrophotometry (Shivhare and Gupta 1991; Jain et al. 1993), immunoassay (Dankwardt 2000), or capillary electrophoresis with ultraviolet (Tomita et al. 1992) or mass spectrometric (Moyano and Galceran 1996) detection. However, although good detection limits can be achieved, as low as 20 ng ml^{-1}, these techniques demand extensive sample treatment and the cost per analysis is high.

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Electroanalytical techniques appear as an attractive, simple and lower cost alternative and have been used in the determination of paraquat with different detection limits (Walcarius and Lamberts, 1996; Pinilla et al. 1993; Lu and Sun, 2000; Navaratne and Susantha, 2000), varying between 10^{-6} to 10^{-9} mol l^{-1}. Electrochemical studies have been carried out at solid electrodes (Monk et al. 1999), mercury electrodes (Walcarius and Lamberts 1996) and at chemically modified electrodes (Lu and Sun 2000).

The electrochemical behaviour of paraquat shows two reversible reduction peaks on sweeping the applied potential in the negative direction. The first, at around -0.7 V vs SCE, is attributed to radical cation formation ($PQ^{2+} \leftrightarrow PQ^{\cdot+}$) and the second at approximately -1.2 V to formation of the neutral species ($PQ^{\cdot+} \leftrightarrow PQ^{0}$) which is probably followed by a chemical dimerisation step (Monk et al. 1999).

Due to the cationic nature of paraquat, the detection limit can be lowered by employing an ion-exchange polymer such as Nafion (Zeng et al. 1996; Lu and Sun 2000) and using adsorption pre-concentration followed by detection using cathodic redissolution (Alvarez et al. 1992). A Nafion-coated glassy carbon electrode was utilized to detect paraquat in river water and urine by differential pulse voltammetry, with an accumulation time of 5.0 min at open circuit. The detection limit was about 0.7 $\mu g\, l^{-1}$and recoveries between 87 and 106% were obtained for spiked river water and urine samples (de Oliveira et al. 2004).

More recently, gold, platinum and carbon fibre microelectrodes have been used in the determination of paraquat in pure electrolyte by square wave voltammetry with detection limits of 3.9, 6.2, and 20.4 $\mu g\, l^{-1}$, respectively (de Souza and Machado, 2003). The authors extended this work to detect paraquat in natural waters and fruit juices with recoveries between 89.5 and 95.0% (de Souza and Machado 2005).

The objective of this work is to investigate the potentialities and exploit the advantages of electrochemical BIA, i.e., with removal of any pre-treatment steps, in the fast-response electroanalytical detection of the pesticide paraquat by square wave voltammetry (SWV) associated with the injection of pesticide into the electrolyte solution.

EXPERIMENTAL

Reagents

The pesticide paraquat was obtained from Sigma-Aldrich. Supporting electrolytes were solutions of 0.01 mol l^{-1} phosphate buffer (pH = 6.9), solutions of acid pH made by mixing the correct proportions of 0.01 mol l^{-1} sodium acetate and 0.01 mol l^{-1} acetic acid. All solutions were made from analytical grade reagents and Millipore Milli-Q water (resistivity $>$ 18 MΩ cm).

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Apparatus

Electrochemical experiments were performed using an Autolab PGSTAT 30 potentiostat with GPES 4.9 software (EcoChemie, Netherlands).

The cyclic and differential pulse voltammetric experiments were carried out in a conventional three electrode electrochemical cell. The working electrode was glassy carbon (diameter 0.5 cm), the auxiliary electrode was of platinum gauze and the reference electrode was saturated calomel (Radiometer K 401).

The electrochemical cell used for BIA analysis was made of perspex that contained a three-electrode system of with an inverted working electrode of glassy carbon (diameter 0.5 cm) with Kel-F sheath at the centre of the cell, as described previously (Brett et al. 1995). A motorized programmable electronic micropipette (Rainin EDP Plus 100) was used to inject the pesticide solution samples, the tip of which was held 2–3 mm above the centre of the working electrode. The cell was filled with inert phosphate buffer supporting electrolyte (pH 6.9).

Experimental Procedure

Initial measurements were made to select the optimal experimental parameters for the analyses. Cyclic voltammetry parameters were scan rate 100 mV s^{-1} in the potential range 0.0 to −1.2 V. Optimised differential pulse voltammetry settings were scan rate 5 mV s^{-1}, scan increment 2 mV, and pulse amplitude 10 mV.

For batch-injection analysis, the chosen square wave frequency was 250 Hz and square wave amplitude 50 mV. The effective scan rate was varied by increasing the increment of potential from 1.0 to 4.0 mV (0.25 to 1.0 V s^{-1}). The volume of the injected sample was varied from 50 to 100 μL, corresponding to injection periods of 2.2 and 1.1 s, respectively. The square wave scan was commenced immediately after the beginning of the injection.

Comparison with the BIA method was made using square wave and differential pulse voltammetry in a conventional three electrode cell (20 ml) using the same electrodes and supporting electrolyte as in the BIA method. The differential pulse voltammograms were recorded using the optimised experimental conditions: scan rate of 5 mV s^{-1}, pulse amplitude 10 mV and scan increment 5 mV. The square wave voltammetry used the same experimental parameters of BIA.

After each measurement, blank phosphate buffer electrolyte was injected to check any effects of adsorption on the electrode. The concentrations of the injected samples of the pesticide solutions were varied up to 10 mg l^{-1} in phosphate buffer electrolyte.

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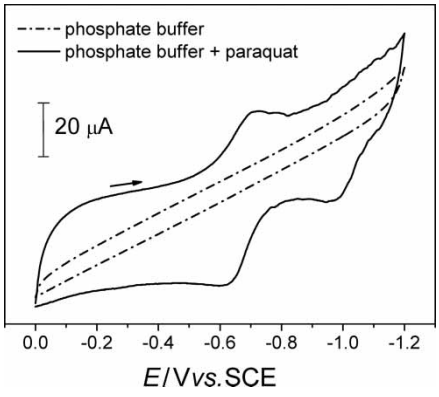

Figure 1. Cyclic voltammetry of a sample of 10 mg l^{-1} paraquat in the potential interval 0 to −1.2 V vs SCE, in 0.1 M phosphate buffer electrolyte (pH 6.9). Scan rate 100 mV s^{-1}.

RESULTS AND DISCUSSION

Optimisation of the Experimental Parameters

A cyclic voltammogram obtained in the potential interval from 0 to −1.2 V in a phosphate buffer solution containing 10 mg l^{-1} paraquat on a glassy carbon electrode is shown in Fig. 1. One reversible redox pair can be observed at −0.65 V, with separation between anodic and cathodic peaks close to 59 mV, and a second reduction peak at −1.0 V, in agreement with the literature (de Souza and Machado 2003 and 2005). The second peak corresponds to a quasi-reversible adsorption process with formation of a neutral species that is followed by a dimerisation step (Monk et al. 1999), and the influence of this in reducing the size of the current peaks was seen in subsequent scans. This cyclic voltammogram is shown without background current subtraction to demonstrate the significant background currents, which can arise on cycling owing to blocking of the electrode surface. In order to avoid adsorption problems in the development of the BIA analytical procedure, the reduction peak centred at −0.65 V was chosen using the potential range from −0.4 to −0.9 V.

Differential pulse (DP) voltammetry, which suffers less from background current effects, was then used to evaluate the effect of pH on the electrochemical reduction of paraquat, from slightly acid (pH = 3.4) to neutral (pH = 7.2) solutions. Over this pH range paraquat is very stable. DP voltammograms, in the potential interval from −0.4 V to −0.9 V, are shown in Fig. 2. The results showed an increase of the peak current close to neutral values of pH, so that the reduction mechanism is favoured by an increase in pH. Nevertheless, there was no variation of the peak potential with pH confirming that protons do not take part in the mechanism.

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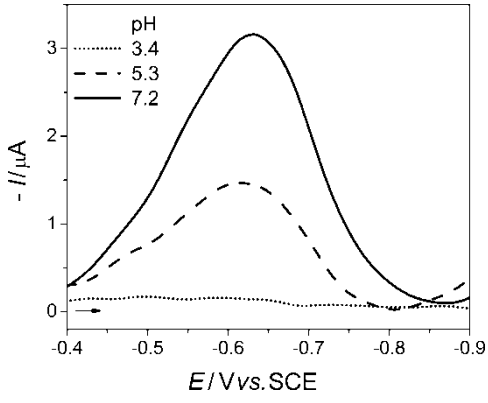

Figure 2. Differential pulse voltammetry of paraquat samples (10 mg l^{-1}) in interval of potential −0.4 to −0.9 V vs SCE. Supporting electrolyte acetic acid 0.01 mol l^{-1} (pH 3.4), sodium acetate 0.01 mol l^{-1} (pH 7.2) and a mixture (pH 5.3). Scan rate 5 mV s^{-1}, scan increment 2.0 mV and pulse amplitude 10 mV.

Batch Injection Analysis

Square wave voltammograms of injected samples of 100 μl of paraquat (10 mg l^{-1}), by batch-injection analysis, at different sweep rates (varying the potential increment) are presented in Fig. 3a. It was found that the best responses with higher peak currents occurred for a frequency of 250 Hz and an increment of potential of 1.0 mV, corresponding to a sweep rate of 250 mV s^{-1}; at lesser frequencies the response was lower. The peak potential occurs at −0.70 V vs. SCE and a small-pre-peak, appearing as a

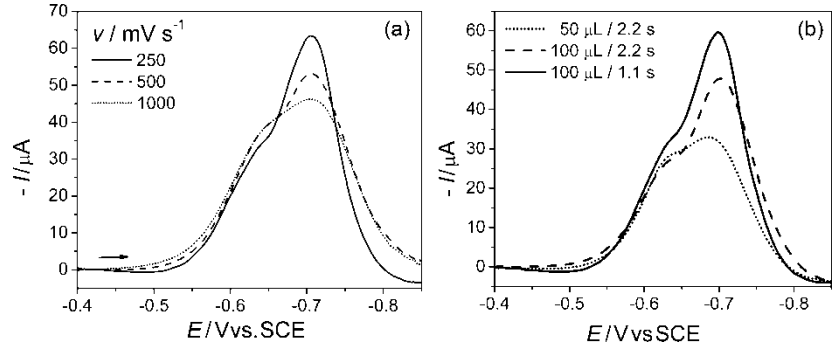

Figure 3. BIA-square wave voltammetry of injected samples of 10 mg l^{-1} paraquat in 0.1 M phosphate buffer supporting electrolyte (pH 6.9) for: (a) different effective scan rates (250, 500, and 1000 mV s^{-1}), varying the potential increment from 1.0 to 4.0 mV. (b) different injection volumes (50 and 100 μl) and injection periods (1.1 and 2.2 s). Potential increment 1.0 mV, frequency 250 Hz.

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shoulder and that can be attributed to adsorbed pesticide, can be seen at ~ -0.62 V.

Figure 3b shows voltammograms obtained from determinations by varying the volume injected and the rate of injection. It was observed that the higher currents occur with larger volumes and higher injection rates, i.e., 100 μl of sample and injection periods of 1.1 s. Since the scan is carried out during the injection period or immediately following it, this would be expected since higher flow rates correspond to higher mass transport, as predicted from wall-jet theory (Brett et al. 1995). The practical deduction from the results in Fig. 3 is that the reduction of paraquat is limited only by sweep rates (i.e., kinetics), the observed current being, in principle, bigger at higher injection flow rates and needing a minimum sample volume to achieve this, which is approximately 100 μl.

Comparison between the results obtained by square wave voltammetry and differential pulse voltammetry in a conventional three-electrode electrochemical cell with batch-injection analysis, is shown in Fig. 4. Although the signal obtained from batch injection analysis is smaller, its quality is higher and the peak is more well-defined with smaller and flatter background currents in the zone of potential outside the paraquat reduction peak. The fact that the signal is smaller, owing to the small amount of analyte used, means that the problems that can arise from electrode fouling—already smaller by using SWV, will be even smaller.

To fully show the potentialities of the technique for the measurement of solutions of paraquat, calibration curves up to 10.0 mg l^{-1} were constructed. Examples of voltammograms over this concentration range are shown in Fig. 5 and a calibration curve in Fig. 6, together with error bars for three

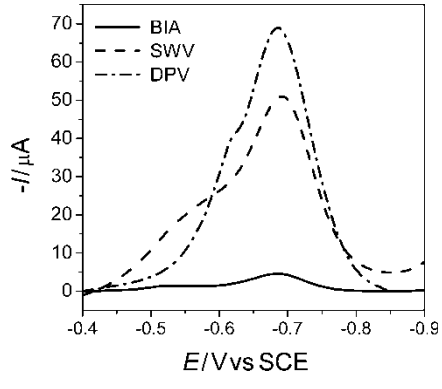

Figure 4. Comparison of square wave and differential pulse voltammetry in a conventional three-electrode cell with BIA), and BIA analysis, of paraquat, concentration 10 mg l^{-1}, in phosphate buffer supporting electrolyte (pH 6.9). Experimental parameters as in Figs 2 and 4.

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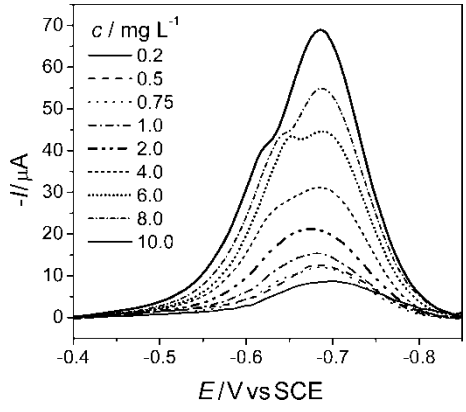

Figure 5. BIA-square wave voltammetry of injected samples of different concentrations of paraquat from 0.2 to 10 mg l^{-1} in 0.1 M phosphate buffer supporting electrolyte (pH 6.9). Other experimental conditions as in Fig. 4.

determinations. It can be seen that there are essentially two response zones. There is a linear range up to 1 mg l^{-1}, with a detection limit that can be estimated as around 20 μg l^{-1}, and a second almost linear one above 1 mg l^{-1}. The latter can be attributed to some adsorption blocking as well to a growing influence of kinetic limitations and is manifested by a larger pre-peak in the voltammograms, almost invisible at lower concentrations, see Fig. 6. Since these are reproducible, as shown by the size of the error bars, the calibration curve can be used to analyse unknown samples.

The power of the batch injection analysis technique in these determinations is its ease of operation and diagnostic capabilities in the field. Most

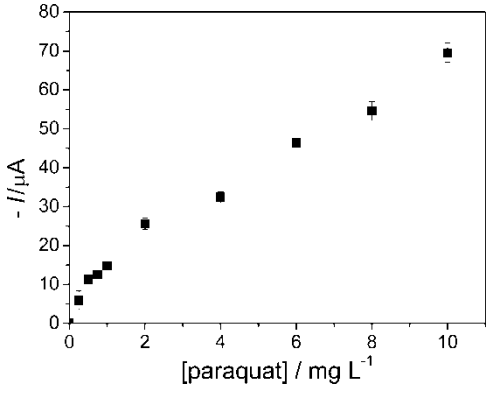

Figure 6. Calibration curve of paraquat for concentrations up to 10 mg l^{-1} vs the measured peak current at −0.7 V. Other experimental conditions as in Fig. 4.

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pesticides will not react at the potentials described. Two scenarios can be envisaged for the analysis of natural samples: first, sample dilution to concentrations below 1 mg l^{-1} before injection and use of the standard addition method and secondly direct measurements of samples containing between 1 and 10 mg l^{-1} paraquat using a pre-recorded calibration curve. It should be noted that, unlike most other electrochemical techniques, no sample dilution is actually required before analyte injection and the response is obtained in a few seconds.

CONCLUSIONS

This paper presents an innovative and powerful tool for the determination of the pesticide paraquat that can be expanded to other electroactive pesticides in environmental sensing. The determination of paraquat by BIA presents many advantages such as extremely fast responses (<2 s), successive measurements without change of supporting electrolyte in the cell, microvolumes of the injected samples (<100 μl) high reproducibility and sufficiently good sensitivity with detection limits in the μg l^{-1} range. Additionally, BIA analysis makes possible determinations in environmental samples in the field, without any pre concentration steps, using portable instrumentation.

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